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DOCUMENT-IDENTIFIER: US 5567417 A
TITLE: Method for inhibiting angiogenesis using heparinase

Abstract Text (1):
Pharmaceutical compositions for delivering an effective dose to a desired site of a heparinase. These compositions are based on the discovery that heparinase alone can inhibit angiogenesis. The effective dosage is dependent not only on the heparinase, but also on the method and means of delivery, which can be localized or systemic. For example, in some applications, as in the treatment of psoriasis or diabetic retinopathy, the inhibitor is delivered in a topical ophthalmic carrier. In other applications, as in the treatment of solid tumors, the inhibitor is delivered by means of a biodegradable, polymeric implant.

Brief Summary Text (2):
Angiogenesis is a fundamental process by which new blood vessels are formed, as reviewed, for example, by Folkman and Shing, J. Biol. Chem. 267 (16), 10931-10934 (1992). It is essential in reproduction, development and wound repair. Under these conditions, angiogenesis is highly regulated, so that it is turned on only as necessary, usually for brief periods of days, then completely inhibited. However, many diseases are driven by persistent unregulated angiogenesis. In arthritis, new capillary blood vessels invade the joint and destroy cartilage. In diabetes, new capillaries invade the vitreous, bleed, and cause blindness. Ocular neovascularization is the most common cause of blindness. Tumor growth and metastasis are angiogenesis-dependent. A tumor must continuously stimulate the growth of new capillary blood vessels for the tumor itself to grow.

Brief Summary Text (3):
Capillary blood vessels consist of endothelial cells and pericytes. These two cell types carry all of the genetic information to form tubes, branches and whole capillary networks. Specific angiogenic molecules can initiate this process. Specific inhibitory molecules can stop it. These molecules with opposing function appear to be continuously acting in concert to maintain a stable microvasculature in which endothelial cell turnover is thousands of days. However, the same endothelial cells can undergo rapid proliferation, i.e. less than five days, during burst of angiogenesis, for example, during wound healing.

Brief Summary Text (4):
A number of proteases have been implicated as key factors in angiogenesis. See, for example, Mignatti, et al., Cell 47, 487-498 (1986) and Rifkin, et al., Acta. Biol. Med. Germ. 40, 1259-1263 (1981), who suggest several enzymes in a proteolytic cascade, including plasminogen activator and collagenase, must be inhibited in order to inhibit angiogenesis.

Brief Summary Text (5):
Under normal conditions, angiogenesis is associated with such events as wound healing, corpus luteum formation and embryonic development, as discussed by Folkman, et al., Science 43, 1490-1493 (1989). However, a number of serious diseases are also dominated by abnormal neovascularization including solid tumor growth and metastases, some types of eye disorders, and rheumatoid arthritis, reviewed by Auerbach, et al., J. Microvasc. Res. 29, 401-411 (1985); Folkman, Advances in Cancer Research, eds. Klein and Weinhouse, pp. 175-203 (Academic Press, New York 1985); Patz, Am. J. Opthalmol. 94, 715-743 (1982); and Folkman, et al., Science 221, 719-725 (1983). For example, there are a number of eye diseases, many of which lead to blindness, in which ocular

neovascularization occurs in response to the diseased state. These ocular disorders include diabetic retinopathy, neovascular glaucoma, inflammatory diseases and ocular tumors (e.g. retinoblastoma). There are a number of other eye diseases which are also associated with neovascularization, including retrolental fibroplasia, uveitis, approximately twenty eye diseases associated with choroidal neovascularization and approximately forty eye diseases which are associated with iris neovascularization. The current treatment of these diseases is inadequate, especially once neovascularization has occurred, and blindness often results.

Brief Summary Text (6):
Key components of the angiogenic process are the degradation of the basement membrane, the migration and proliferation of capillary endothelial cell (EC) and the formation of three dimensional capillary tubes. The normal vascular turnover is rather low: the doubling time for capillary endothelium is from 50-20,000 days, but it is 2-13 days for tumor capillary endothelium. The current understanding of the sequence of events leading to angiogenesis is that a cytokine capable of stimulating endothelial cell proliferation, such as fibroblast growth factor (FGF), causes release of collagenase or plasminogen activator which, in turn, degrade the basement membrane of the parent venule to facilitate in the migration of the endothelial cells. These capillary cells, having `sprouted` from the parent vessel, proliferate in response to growth factors and angiogenic agents in the surrounding to form lumen and eventually new blood vessels. Thus, inhibition of angiogenesis can occur at any of the above key junctures. A chemical agent which prevents the continued spread of vascularization could have broad applicability as a therapy for those disease in which neovascularization plays a prominent role.

Brief Summary Text (10):
Although a number of these studies have focused on the role of heparin-like molecules in neovascularization, little is known about the role of heparin-degrading enzymes in neovascularization. Heparin-like molecules such as heparin and heparan sulfate bind several cytokines, which are angiogenic, and modulate their function either by stabilizing them or by controlling their bioavailability, as reported by Folkman and Shing, J. Biol. Chem. 267, 10931-10934 (1992). These molecules have been shown by Klagsbrun and Baird Cell 67, 229-231 (1991), to act as low affinity receptors on cell surfaces and to facilitate growth factor activity and receptor binding.

Brief Summary Text (11):
These observations suggest that enzymes which degrade heparin-like molecules can play a role in modulating neovascularization. Far less is known about the direct role of heparinase on the angiogenic process than is known about that of its substrate, heparin.

Brief Summary Text (12):
Heparin lyases are a general class of enzymes that are capable of specifically cleaving the major glycosidic linkages in heparin and heparan sulfate. Three heparin lyases have been identified in Flavobacterium heparinum, a heparin-utilizing organism that also produces exoglycouronidases, sulfoesterases, and sulfamidases that further act on the lyase-generated oligosaccharide products (Yang, et al. J. Biol. Chem. 260, 1849-1857 (1987); Galliher, et al. Eur. J. Appl. Microbiol. Biotechnol. 15, 252-257 (1982). These lyases are designated as heparin lyase I (heparinase, EC 4.2.2.7), heparin lyase II (heparinase II, no EC number) and heparin lyase III (heparitinase EC 4.2.2.8). The three purified heparin lyases differ in their capacity to cleave heparin and heparan sulfate: Heparin lyase I primarily cleaves heparin, heparin lyase III specifically cleaves heparan sulfate and heparin lyase II acts equally on both heparin and heparan. Several Bacteroides sp. (Saylers, et al. Appl. Environ. Microbiol. 33, 319-322 (1977); Nakamura, et al. J. Clin. Microbiol. 26, 1070-1071 (1988)) also produce heparinases. A heparinase has also been purified to apparent homogeneity from an unidentified soil bacterium by Bohmer, et al.J. Biol. Chem. 265, 13609-13617 (1990).

Brief Summary Text (14):
It is an object of the present invention to provide pharmaceutical compositions, and method of use thereof, based on heparinases, for the treatment of diseases involving abnormal angiogenesis.

Brief Summary Text (16):
It is another object of the present invention to provide topical and controlled release pharmaceutical compositions, and methods of use thereof, based on heparinases, for inhibition of angiogenesis.

Brief Summary Text (18):
Pharmaceutical compositions for delivering an effective dose of a heparinase to a desired site. These compositions are based on the discovery that heparinase alone can inhibit angiogenesis. The heparin-degrading enzymes heparinases I and III have now been demonstrated to inhibit both neovascularization in vivo and proliferation of capillary endothelial cells mediated by basic fibroblast growth factor in vitro, and caused dramatic loss in binding of basic fibroblast growth factor to these cells. Heparinase II did not inhibit neovascularization in vivo, had marginal effect on endothelial cell proliferation and the binding of basic fibroblast growth factor to these cells, but is very useful in the alteration of smooth muscle cell proliferation.

Brief Summary Text (19):
The effective dosage for inhibition of angiogenesis in vivo, defined as inhibition of capillary endothelial cell proliferation and migration and blood vessel ingrowth, is extrapolated from in vitro inhibition assays. In vitro assays have been developed to screen for inhibition of angiogenesis. Events that are tested include proteolytic degradation of extracellular matrix and/or basement membrane; proliferation of endothelial cells, migration of endothelial cells, and capillary tube formation. Effective dosages range from 10 nM to 100 nM heparinase I or III for inhibition of capillary endothelial cell proliferation and between 1 .mu.g and 4 .mu.g heparinase I or III for inhibition of localized angiogenesis. The effective dosage is dependent not only on the heparinase, but also on the method and means of delivery, which can be localized or systemic. For example, in some applications, as in the treatment of psoriasis or diabetic retinopathy, the inhibitor is delivered in a topical ophthalmic carrier. In other applications, as in the treatment of solid tumors, the inhibitor is delivered by means of a biodegradable, polymeric implant.

Drawing Description Text (2):
FIGS. 1a, 1b, 1c and 1d are micrographs showing inhibition of angiogenesis by heparinase in the chick chronic membrane assay. FIG. 1a is the CAM with heparinase I containing disk: 100% of the eggs tested (n=12) of several different batches of heparinase I, had avascular zones; FIG. 1b is a normal CAM containing an empty methylcellulose disk; FIG. 1c are histological sections of day 8 normal CAMs .times.800; and FIG. 1d heparinase I treated CAM.

Detailed Description Text (2):
It has been discovered that heparinases directly inhibit neovascularization. It has also been observed that heparinases of different substrate specificity differentially affect BCE proliferation and bFGF binding to these cells. For example, the substrate specificities of heparinases I, II and III from F. heparinum may control either the availability or the removal of unique heparin fragments essential for neovascularization. Heparinase III acts at the more "heparan sulfate-like regions" of the endothelial cell polysaccharide, leaving behind intact bFGF binding sites, and heparinase I cleaves the "heparin-like regions" of the polysaccharide containing the bFGF binding sites; thus both enzymes differentially modulate the availability of specific bFGF binding sites required for bFGF-mediated BCE proliferation, as described by Nader, et al. Proc. Natl. Acad. Sci. U.S.A. 84, 3565-3569 (1987); Lindblom, et al. Biochem J. 279, 821-829 (1991); and Turnbull, et al. J. Biol. Chem. 267, 10337-10341 (1992). However, since heparinase II non-specifically cleaves the polysaccharide primarily into disaccharides, this enzyme probably does not hit enough sites to alter heparan sulfate structure significantly enough to affect bFGF binding and activity.

Detailed Description Text (3):
In complete contrast to the observations described herein, it has been suggested by Folkman, J. & Shing, Y. J. Biol. Chem. 267, 10931-10934 (1992), that enzymes such as heparinases, which degrade ECM structural components, have a role in aiding the release of growth factors sequestered in the matrix and facilitating EC proliferation and neovascularization in vivo. For example, it has been proposed by Vlodavsky, et al. Cancer Met. Rev. 9, 203-226 (1990), that heparinases degrade the basement membrane

serving as a reservoir for bFGF to initiate tumor or wound neovascularization.

Detailed Description Text (4):
As described herein, however, these enzymes appear to have another role wherein they inhibit neovascularization by depleting the low affinity receptors that are critical for bFGF mediated proliferation of endothelial cells and hence neovascularization. In addition to bFGF, several endothelial cell cytokines have been found by Gitay-Goren, et al. J. Biol. Chem. 267, 6093-6098 (1992) and Folkman, J. & Klagsbrun, M. Science 235, 442-447 (1987), to bind and be modulated by heparin-like molecules; thus heparinases could act on wide spectrum of heparin-dependent angiogenic factors. The mechanism described here for specific heparinase-mediated inhibition of bFGF binding and activity on BCEs is believed to be reflective of a general system for the control of capillary endothelial cell proliferation and neovascularization and therefore subject to regulation using any molecule having heparinase-like activity.

Detailed Description Text (7):
A variety of heparinases are produced in bacteria, especially Flavobacterium heparinum, which produces three distinct heparinases, heparinase I, heparinase II, and heparinase III. As described herein, heparinase I and heparinase III are potent inhibitors of neovascularization. In the preferred embodiment, only heparinase purified of other activities or contaminants is used. Although heparinase is commercially available, it is preferred to use a more highly purified form, especially when derived from F. heparinum, since the bacteria produces a number of enzymes other than heparinase, such as chondroitinase, as well as the three very different forms of heparinase. Reports of studies conducted with the commercially available forms of heparinase have contained conflicting results and non-reproducible data, presumably due to contamination with other materials.

Detailed Description Text (8):
As used herein, "heparinase" includes the enzymes derived from natural sources, as well as those which have been modified naturally or synthetically to alter their binding affinity and/or catalytic activity.

Detailed Description Text (14):
Enzyme assays and absorbance measurements were done on a UV 160 spectrophotometer from Shimadzu connected to a Fisher Scientific Isotamp model 9100 refrigerated circulating water bath. Fermentations were performed in a two-liter stirred tank fermenter from Applikon. Centrifugation was done on a Sorvall RC-5 refrigerated centrifuge in a GSA rotor from Du Pont. HPLC was performed using a LDC Milton-Roy Constametric IIIG pump, a Rheodyne 7125 injector, a Jule Linear Gradient Former, and an ISCO model UA-5 absorbance monitor with a 280-nm filter. The hydroxylapatite HPLC column 1.times.30 cm connected in series with a 1.times.5 cm guard column was from Regis, the Mono-S FPLC column was from Pharmacia LKB Biotechnology Inc., the C.sub.18 column was from Vydac, and the Bio-Sil gel permeation HPLC column was from Bio-Rad. The capillary zone electrophoresis system and the silica capillaries were from Dionex. The Mini-Protein II electrophoresis chamber, a model 1405 horizontal electrophoresis cell, and a model 1420B power source were from Bio-Rad. The tube gel electrophoresis equipment was from E-C Apparatus Corp. The precast agarose IEF gels were from Iso-labs, and the prestained molecular weight markers and the Rapid Coomassie.TM. stain were from Diversified Biotech. The Bio-Gel HT hydroxylapatite was from Bio-Rad and the QAE-Sephadex was from Sigma. Pressure filtration units and 25- and 43-mm PM-10 filters were from Amicon. Heparin (porcine mucosal sodium salt) was from Celsus, heparan sulfate, dermatan sulfate, and chondroitin sulfate A, C, D, and E were from Seikagaku. Bovine serum albumin, lactose, protamine (free base), bromphenol blue, naphthol red, cytochrome c (bovine heart type VA), hyaluronic acid, CAPS, bis-Tris, HEPES, TES, dithiothreitol, MOPS, mercaptoethanol, iodoacetamide, and trypsin were for Sigma. The Coomassie reagent for the protein assay was from Bio-Rad. All water used in reagents was deionized and distilled in glass.

Detailed Description Text (17):
Fermentation and Enzyme Recovery

Detailed Description Text (28):
The 510 ml of supernatant containing 15.6 mg/ml protein, used directly without freezing, was divided equally into four 250 ml polypropylene centrifuge containers and

placed in an ice bath. Dry hydroxylapatite (HA) (20 g) was added to each container, gently stirred, lightly compacted by centrifugation at 1000.times. g for 2 min at 4.degree. C., and the supernatant was decanted away from the HA matrix. The HA-bound protein was then resuspended in buffers having increasing concentrations of sodium phosphate and sodium chloride and recompacted by centrifugation. The supernatants were again decanted away from the matrix and assayed for enzyme activity and protein concentration. The buffers used to wash the HA matrix were prepared by mixing a solution of 10 mM sodium phosphate buffer at pH 6.8, with a solution of 250 mM sodium phosphate buffer at pH 6.8, containing 500 mM sodium chloride in ratios of 6:0, 5:1, 4:2, 3:3, 2:4, and 0:6 (v/v) at 4.degree. C. The protein supernatant solutions were placed in dialysis tubing having a molecular weight cut-off of 14,000 and dialyzed overnight at 4.degree. C. against 50 mM sodium phosphate buffer at pH 7.0.

Detailed Description Text (46):
II. Assays for determining effectiveness and effective concentration of angiogenesis inhibitors in vivo

Detailed Description Text (47):
Assays were used to test heparinase for endothelial cell proliferation, FGF binding to endothelial cell receptors, and inhibition of angiogenesis in vivo.

Detailed Description Text (48):
The essential elements of these assays include using endothelial cells as the assay target and stimulation of the endothelial cells with known angiogenesis factors to compare a putative inhibitor's effect in unstimulated, as compared to stimulated, endothelial cells. Endothelial cells that can be used include capillary endothelial cells and umbilical vein endothelial cells. Factors which can be used to stimulate the endothelial cells include acidic or basic fibroblast growth factor.

Detailed Description Text (49):
The following assays are based on assays described in the prior art that have been modified for the purpose of screening heparinase for inhibition of angiogenesis in vivo.

Detailed Description Text (59):
Capillary EC proliferation in response to an angiogenic stimulus is a critical component of neovascularization, as discussed by Ausprunk and Folkman, J. Microvasc. Res. 14, 153-65 (1977). By utilizing the specific cells involved in angiogenesis, and stimulating them with known angiogenesis factors, in this case basic fibroblast growth factor (bFGF), as reported by D'Amore and Klagsbrun, J. Cell Biol. 99, 1545-1549 (1984), it is possible to mimic the angiogenesis process in vitro. This type of assay is the assay of choice to demonstrate the stimulation of capillary EC proliferation by various angiogenic factors and inhibitors, as reviewed by Shing, et al., Science 223, 1296-1298 (1984).

Detailed Description Text (72):
Confluent capillary EC monolayers were prepared by plating 1.times.10.sup.5 cells per 4 cm.sup.2 well (12-well plates, Costar, Cambridge, Mass.) in DMEM, 10% calf serum (1 ml/well) and incubating at 37.degree. C. for 3-5 days. To initiate enzyme treatment, the medium was removed, the cells washed one time with DMEM (1 ml/well), and heparinase added at concentrations of 0, 0.1, 1, 10, or 100 nM in 0.25 ml DMEM containing 5 mg/ml BSA. Enzyme treatment was carried out at 37.degree. C. for 30 min. When treatment was completed the enzyme medium was removed and the monolayers washed twice (1 ml/well/wash) with cold (4.degree. C.) binding buffer (DMEM, 25 mM HEPES, 0.5% gelatin), and then incubated for 10 min at 4.degree. C. in 0.5 ml binding buffer to precool the cells. .sup.125 I-bFGF (5 ng, 0.66 nM, (1.25.times.10.sup.5 to 5.times.10.sup.5 cpm) was then added. The plates were incubated at 4.degree. C. for 2 h, at which point the binding buffer was removed and each well washed 3 times with cold binding buffer (1 ml/well/wash). The amount of a .sup.125 I-bFGF bound to HSPG and receptor was determined sequentially in each culture using a modification of the salt/acid washing technique of Moscatelli, D. J. Cell. Physiol. 131, 123-130 (1987) and Nugent, M. A. & Edleman E. Biochemistry 31, 8876-8883 (1992). .sup.125 I-bFGF bound to HSPG was released by exposure to high salt buffer (2M NaCl, 20 mM HEPES, pH 7.4; 0.5 ml/well for 5 s), and the .sup.125 I-bFGF bound to receptors was extracted by incubation of the monolayers in low pH buffer (2M NaCl, 20 mM sodium acetate, pH 4.0;

0.5 ml/well; 5 min) followed by a wash with the same buffer (0.5 ml/well). .sup.125 I-bFGF was determined in all samples by counting in a 1272 CliniGamma gamma counter (LKB Nuclear, Gaithersburg, Md.). The .sup.125 I-bFGF bound that was not competed by an excess (5 .mu.g; 55 nM) of unlabeled bFGF was defined as nonspecific and was subtracted from the experimental points. The number of cells attached to the culture plates before and after the salt and acid washes was similar.

Detailed Description Text (78):
While heparinase II has a marginal effect on the BCE proliferation in the concentration ranges of between 10 and 100 nM where heparinase III and I dramatically inhibit BCE proliferation, all three enzymes show similar effects in bFGF binding to these cells. In the 10 to 100 nM concentration range, almost all heparan sulfate binding and about 90, 85 and 80% of the receptor binding is eliminated for heparinases III, I and II, respectively.

Detailed Description Text (79):
In summary, the effect of heparinase was first tested on the proliferation of capillary endothelial cells (ECs) in vitro in the presence and absence of bFGF, a potent stimulator of EC proliferation and angiogenesis. Heparinase I was a powerful inhibitor of FGF-stimulated capillary EC proliferation with an IC.sub.50 of 60 nM. At very low concentrations of heparinase (14.2 pM and 142 pM) this enzyme caused small but reproducible potentiation of FGF stimulation.

Detailed Description Text (83):
As noted above, several types of heparinase can be used as described herein to inhibit angiogenesis. The effective dosage will depend on the purity and origin of the heparinase, as well as the type, for example, heparinase I versus heparinase II. The preferred enzymes are heparinase I and heparinase III from F. heparinum. Pharmaceutical compositions are prepared using heparinase as the active agent to inhibit angiogenesis based on the specific application. Application is either topical or localized. For topical application, the purified heparinase is combined with a carrier so that an effective dosage is delivered, based on the desired activity, i.e. ranging from an effective dosage, for example, of 1 and 4 .mu.g heparinase (with 100% inhibition of angiogenesis at between 3 and 4 .mu.g) to prevent localized angiogenesis to between 10 and 100 nM to inhibit capillary endothelial cell proliferation, with 100% inhibition at approximately 80 nM heparinase I or III. A topical heparinase composition is applied to the skin for treatment of diseases such as psoriasis. The carrier may be in the form of an ointment, cream, gel, paste, foam, aerosol, suppository, pad or gelled stick.

Detailed Description Text (85):
Heparinase compositions for local or regional administration, for example, into a tumor, will generally include an inert diluent, and will be administered in an amount effective to inhibit angiogenesis, as noted above. Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Commercially available heparinase preparation come either lyophilized or in sterile water or buffer, but are rarely of the purity that is desired for this application.

Detailed Description Paragraph Table (1):
TABLE 1 _____ Capillary Endothelial Cell Proliferation Assay demonstrating the Effect of heparinases. 0.005 0.05 0.5 5.0 Enzyme .mu.g/ml .mu.g/ml .mu.g/ml .mu.g/ml _____ Heparinase I +12% no effect -25% -100% Heparinase II -4% -8% no effect -20% Heparinase III +12% +45% -60% -95% _____

Other Reference Publication (6):
Ausprunk, D. H., et al., "Migration and Proliferation of Endothelial Cells in

Preformed and Newly Formed Blood Vessels During Tumor Angiogenesis", J. Microvasc. Res., 14:153-65 (1977).

Other Reference Publication (11):
Folkman, J. et al., "Minireview-Angiogenesis", J. Biol. Chem., 267(16):10931-10934 (1992).

Other Reference Publication (13):
Folkman, J., et al., "Angiogenesis Inhibition and Tumor Regression Caused by Heparin or a Heparin Fragment in the Presence of Cortisone", Science, 221:719-725 (1983).

Other Reference Publication (15):
Folkman, J., "Tumor Angiogenesis", Advances in Cancer Research, Academic Press, Inc., New York, 43:175-203 (1985).

Other Reference Publication (16):
Folkman, J., et al., "Control of Angiogenesis With Synthetic Heparin Substitutes", Science, 243:1490-1493 (1989).

Other Reference Publication (46):
Taylor, S., et al., "Protamine is an Inhibitor of Angiogenesis", Nature (London), 297:307-312 (1982).

Other Reference Publication (50):
Vlodavsky, I., et al., "Extracellular Matrix-resident Growth Factors and Enzymes: Possible Involvement in Tumor Metastasis and Angiogenesis", Cancer and Metastasis Reviews, 9:203-226 (1990).

CLAIMS:

1. A composition inhibiting angiogenesis comprising an effective amount of a heparinase selected from the group consisting of heparinase I and heparinase III from Flavobacterium heparinum in a pharmaceutically acceptable carrier for topical administration to inhibit angiogenesis at a selected site in a non-heparinized patient in need of treatment thereof, wherein the carrier delivers to a site where angiogenesis is to be inhibited between approximately one and four .mu.g heparinase or a concentration of between 10 and 100 nM heparinase.

5. A method for inhibiting angiogenesis comprising:

administering to a non-heparinized patient in need of treatment thereof to inhibit angiogenesis an effective amount of a heparinase selected from the group consisting of heparinase I and heparinase III from Flavobacterium heparinum in a pharmaceutically acceptable carrier to a site where inhibition of angiogenesis is desired.

11. The method of claim 5 wherein an effective dose at the site where angiogenesis is to be inhibited is between approximately one and four .mu.g heparinase or a concentration of between 10 and 100 nM heparinase.